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Synthesis of potentially bioactive Diels-Alder 5-HydroxymethylFurfural adducts

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Abstract

In the past years, we have come to experience some negative effects of long term world reliance on fossil derived production. The effects on the environment are extensive observable and, on top of this, chaotic oil market prices have been causing great conc
In the current context, pressure to develop new methods of production derived from renewable sources has extended to all areas of big industry. Chemical Industry is no exception. The majority of carbon based starting molecules are still obtained from fossil feedstock, but now we have been experiencing the increase of new ways for production for these starting materials, leading to a *greener* chemistry. Here is where 5-hidroxymethylfurfural (5-HMF) comes in to picture. This molecule has been known for some time, but only recently was achieved a reliable, efficient method for production that allows us to consider 5-HMF as a promising starting molecule in synthesis. 5-HMF comprises a furan ring, a structure that has been studied as a substrate for a type of synthetic useful reaction: the inverse electron demand Diels-Alder (IEDDA) reaction. In order to explore the reactivity of 5-HMF type structures in IEDDA, we devised a small compound family composed of several HMF derivatives, acting as dienes, and then tested the formation of DA adducts using 2,3-dihydrofuran (DHF) as dienophile. We optimized some of the reactions leading to the small family of substrates, screening parameters such as solvent used and reaction time. Although the isolation of most of the final adducts was not achieved, the reactivity was observed and can be improved in order to be applied to biological systems, with possible applications in areas such as click chemistry.

Keywords: HMF; 5-hydroxymethylfurfural; inverse-electron demanding Diels-Alder reaction; HMF derivatives

Resumo

Nos últimos tempos tem-se vindo a assistir ao impacte negativo da dependência global de meios de produção em fontes fósseis. Os efeitos deste uso prolongado no Ambiente são extensos e inegáveis. As fontes fósseis escasseiam e o têm afectado os mercados internacionais. Neste contexto actual, todas as grandes industrias, incluindo a industria química, têm vindo a ser pressionadas para abandonarem meios de produção que assentam em fontes fósseis, e a desenvolverem novos métodos que se baseiem em fontes de carbono renováveis.

5-hidrometilfurfural (5-HMF) é uma molécula obtida através da desidratação da frutose e já há algum tempo que tem vindo a ser estudada como reagente em processos sintéticos. Contudo só há relativamente pouco tempo foi desenvolvido um método de produção eficiente desta molécula, capaz de rendimentos ao nível industrial. Olhando para a sua estrutura molecular podemos encontrar um anel furano, capaz de diversos tipos de reactividade, incluindo um de particular interesse sintético: formação de novas ligações C – C através de reação de Diels-Alder (DA). Criou-se então uma biblioteca de compostos derivados do 5-HMF, reagindo como dienos, e a sua rectividade foi testada, usando 2,3-dihidrofurano (2,3-DHF) como dienófilo. Apesar dos aductos finais da reação não terem sido isolados com sucesso, o conceito foi provado, abrindo portas para que este tipo de reactividade seja mais bem estudada e explorada, podendo possivelmente ser aplicada a sistemas biológicos e tendo por isso interesse na área das ciências farmacêuticas.

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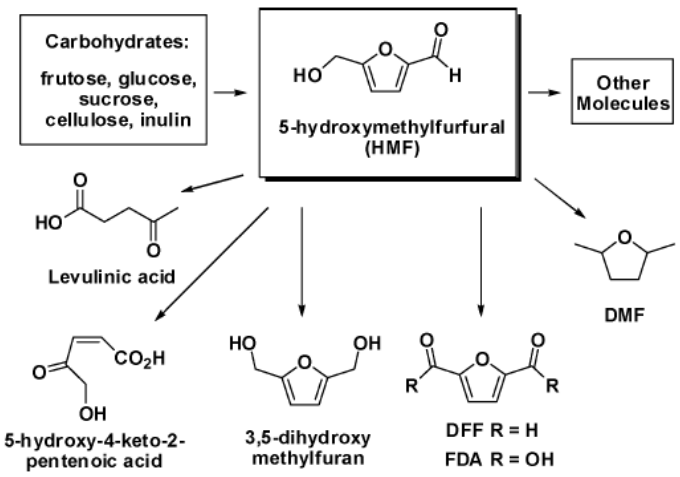
1. Introduction

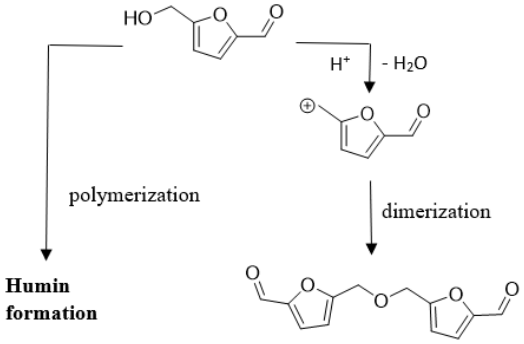
HMF and its potential as a building block

The main source of functionalized carbon skeletons for use in Chemical industry is still based on fossil fuel reservoir. However, with the rise in oil prices(1) and the increasing concern about environment changes, the society has been changing its paradigm to push sustainable development upon its big industries(2). We have seen this changes in the Energy industry, where wind and solar power systems are gaining major relevance in today's public consumption, and in fuel industry, with electric and bio based powered vehicles being considered as one of the options for future transportations(3)(4). The Chemical industry is no different with the rise of such concepts as green chemistry where not only the carbon sources but also the transportation, safety and waste treatment are the focus. Up to 30% of raw chemical materials are estimated to be produced from biomass in the year 2025(3) so this is the new reality in which modern chemistry will be built upon. It is within this new-found approach that we find 5–hydroxymethylfurfural (HMF), a promising molecule once described as a "sleeping giant"(5) that has been studied by the scientific community for some time now(6)(7). It can be obtained from redly available sources like fructose, glucose and possibly cellulose and its of great importance in the production of dimethylfuran (DMF) a biofuel, as well as other important molecules such as 5-hydroxy-4-keto-2-pentenoic acid, 2,5-diformylfuran (DFF), dihydroxymethylfuran, 2,5-furanidicarboxylic acid (FDA) and levulinic acid(7)(8)(Scheme 1). HMF has also been found to affect the growth and metabolism of several *Saccharomyces cerevisiae* strains and demonstrated anti-sickling effect by forming a Schiff-base adduct with HbS and increasing oxygen affinity of sickled cells in hypoxic conditions(9), showing not only building block but also biological potential. In spite of all its promising characteristics, HMF is not without some troubling issues. The presence of side reactions, mainly humin formation (Scheme 2), characteristic of saccharide chemistry, associated with different reactivity pathways with complementary reaction conditions (base catalyzed glucose isomerization *vs* acid catalyzed fructose dehydration) present synthetic obstacles that must be addressed(5). The isolation and purification of HMF is also an arduous process due to the molecule high solubility in aqueous media and polar solvents, its low melting point (30 – 34°C) and vapor pressure (114 - 116°C/ 1 mbar) and its relative instability.

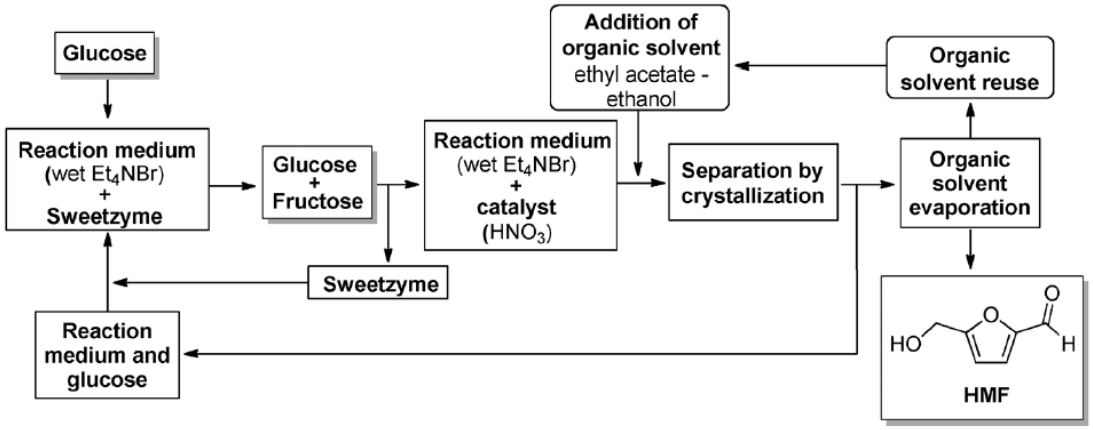


Scheme 1

Simeonov *et al*.(10)(11) addressed this problems by developing an integrated method for production of HMF from glucose using ammonium salts such as tetrapropylammonium bromide (TPAB) or tetraethylammonium bromide (TEAB) as the reaction media and then crystallizing this media, allowing HMF isolation trough organic solvent evaporation. Regarding the difficult glucose-fructose isomerization, this step was achieved using an enzyme, sweetzyme, followed by chemical dehydration. Overall this process affords yields up to 87% and final HMF purity of >99% and combined with the fact that reaction medium and sweetzyme can be efficiently reused makes this a possible method for HMF production in large scales (Scheme 3).



Scheme 2 - Possible side reactions for HMF. Humin formation leads to extremely insoluble polymers.



Scheme 3 - The integrated approach for the production and isolation of HMF from glucose.

The Diels-Alder Reaction

Looking at the molecular structure of HMF we can identify three possible reaction sites: its hydroxymethyl group, the furan ring, comprising two conjugated π bonds, and its formyl group. Regarding its hydroxymethyl and formyl groups, various transformations have been described such as selective oxidations on both sites(7), reductive amination of the formyl and other powerful synthetical tools like the witting reaction(6). Oxidation of the furan ring is also possible under photo-oxidation conditions, however one can wonder if the π conjugated system can be explored, namely through Diels-Alder (DA) reaction.

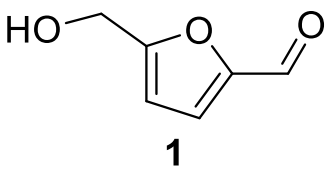


Figure 1 – Reactive structure of HMF.

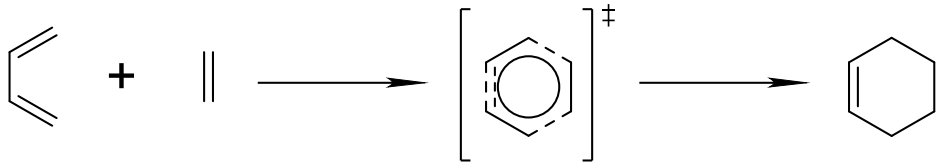


Figure 2 - Diels-Alder reaction scheme.

This type of chemical transformation is a powerful synthetical tool, forming two new σ bonds in a relative well establish, robust chemistry. DA reactions are a type of cycloaddition, more specifically a [4 + 2] cycloaddition, which, according to frontier molecular orbital (FMO) theory, can be divided into three types, depending on the energy gap (ΔE) between the HOMO and LUMO of the diene and dienophile. Type I, designated normal DA, when the $HOMO_{diene}$ - $LUMO_{dienophile}$ gap is smaller than $HOMO_{dienophile}$ - $LUMO_{diene}$, type II, neutral DA, when HOMO-LUMO separations are equivalent, and type III, inverse electron demand Diels-Alder (iEEDA), where the reactivity is governed by $HOMO_{dienophile}$ - $LUMO_{diene}$ separation. This classification allows the description of the reaction in a qualitative way, as well as semiquantitative. Substituent groups in the two reactants govern the energy of this orbitals and it is known that electron withdrawing substituents lower both HOMO and LUMO, electron donating substituents raise both HOMO and LUMO and extra conjugation lowers LUMO and raises HOMO(12). Solvent also seems to play a significant role, especially when the solvent is water. This type of cycloadditions has been found to occur faster when the reaction medium is partial on completely on water, even if the dissolution of reactants is not complete. This effect seems to be linked to the formation of hydrophobic pockets caused by the surrounding of reactant molecules by water molecules, as well as charge stabilization and dipolar effects(13). DA reactions are also an interesting possible tool in domains such as click chemistry(14)(15)(16), for it demonstrates a variety of characteristics appealing for this concept: has theoretical high rates in water, can be selective and high yielding and it is an overall clean and complete reaction.

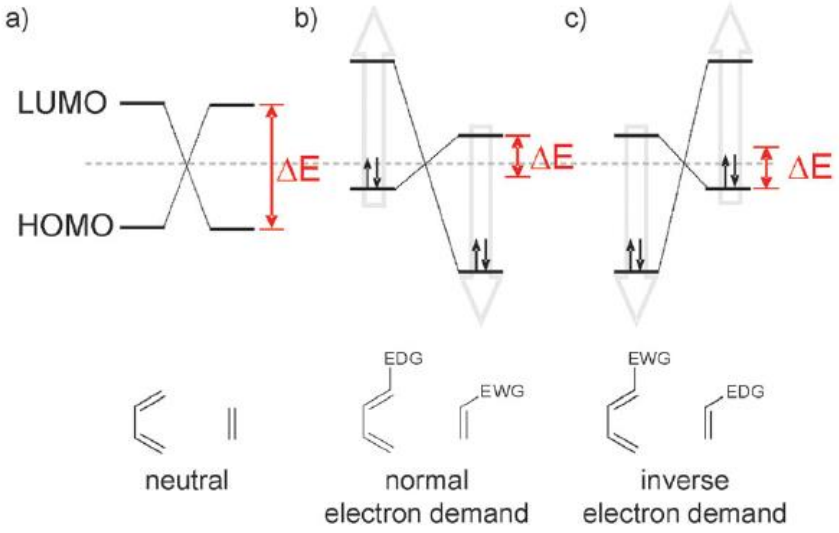


Figure 3 - Frontier orbital model of (a) neutral, (b) normal electron demand and (c) inverse electron demand Diels-Alder additions.

Biological assays and its importance in studying biological systems

In order to estimate and test the activity of chemical compounds in a biological system we used biological assays, also called bioassays. In this type of biological experiment, the objective is to compare the potencies of response, that can be measured, between the tested molecule and a standard which is known for that given response. Bioassays can be performed in living beings, usually on an advanced stage of research, or *in vitro* using cell cultures, the preferred method in initial stages of drug discovery. There are several types of assay depending on the information available and the type of response that being measured, but the three main ones are the direct assay, when the response is specifically produced and can be directly measured, the indect assay, when the relation between dose and response need to be ascertained and a quantal assay, when the response is in the form of "all or nothing", being produced by a threshold effect. To be scientifically valid, these biological assays have to be well constructed, the detection and measuring method has to be sensitive and specific enough to a given response and the response itself has to be well established. These criteria are sometimes difficult to reach, distinctively in the early stages of research, but this kind of biological experiments remains a powerful tool in understanding the potentially activity of substances in biological systems(17).

2. Objective

In this research project, we explore the potential for inverse electron demand Diels-Alder reaction of HMF and its derivatives, mainly hydrazones and oximes, in order to create possible future applications, both in direct biological activity as well as chemical applications on "click chemistry". For this purpose, we established a small compound family derived from HMF and its reaction with various amines, hydrazines, hydroxilamines and Meldrum's acid (2,2-dimetyl-1,3-dioxane-4,6-dione), creating structures with lower electron density in the furan ring, serving as dienes, in order to test kinetics rates and reactivity with a dienophile (2,3-dihydrofuran), proving the concept of inverse Diels-Alder in these compounds.

3. Experimental section

Materials

All reagents were purchased from Sigma-Aldrich and Alfa-Aesar. Methanol and dichloromethane were dried from calcium chloride and distilled at atmospheric pressure. Acetonitrile was dried from Na and distilled at atmospheric pressure prior to use. The remaining solvents were used without further purification. 5-HMF was synthetized using the method reported here(11). The imines derived from 5-HMF were synthetized and isolated by João Ravasco. The acetized HMF was synthetized by Rafael Gomes.
The reaction mixtures were analyzed by TLC using ALUGRAM® SIL G/UV254 from NM (Ref. 81833, silica gel 60), and visualization of TLC spots was effected using ultraviolet (UV) and phosphomolybdic acid solution stain. NMR spectra were recorded on a Bruker Fourier 300 NMR spectrometer (1H 300 MHz; 13C 100.61 MHz). ^{1}H and ^{13}C chemical shifts (δ) are expressed in ppm (parts per million) and are relative to the corresponding resonance of non-deuterated solvent. Coupling constants (*J*) are reported in Hz. HPLC analysis was performed on a VWR Hitachi apparatus with a diode array detector L-2455 coupled to a pump L-2130 and using a KROMASIL 100 SIL 5.0 column, manual injector with 20 µL loop.

Reaction procedures

Synthesis of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) at room temperature

To a solution of malonic acid (1.0 g, 9.60 mmol) in acetone (0.7 mL, 9.53 mmol), acetic anhydride (1 mL, 10.59 mmol) was added, followed by addition of catalytical sulfuric acid (2 drops). The mixture was allowed to stir at room temperature and the reaction was followed by TLC until completion. The reaction was quenched with a saturated solution of $NaHCO_3$ until pH 4. The desired product was extracted in ethyl acetate, the organic was dried with Na_2SO_4. The product was concentrated in vacuum.

Synthesis of 5-((5-(hydroxymethyl)furan-2-yl)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione

To a solution of 2,2-dimethyl-1,3-dioxane-4,6-dione in water (0.1 M), 5-HMF (1 equiv.) was added. The mixture was allowed to stir for 4 h at 75ºC. The desired molecule was extracted in dichloromethane, the organic layer was dried with Na_2SO_4. The product was concentrated in vacuum. The solid product was recrystallized using MTBE and hexane.

Synthesis of (5-((2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)methyl)furan-2-yl)methyl acetate

To a solution of 2,2-dimethyl-1,3-dioxane-4,6-dione in water (0.1 M), acetylated HMF (1 equiv.) was added. The mixture was allowed to stir for 23 h. at 75ºC. The desired molecule was extracted in ethyl acetate, the organic layer dried with Na_2SO_4. The product was concentrated in vacuum. Separation was accomplished by preparative thin layer chromatography, using a mixture of Hexane/MTBE 8:2 as eluent.

Synthesis of (E)-(5-((2-phenylhydrazono)methyl)furan-2-yl)methanol

To a solution of phenylhydrazine in ethanol (0.1 M), 5-HMF (1 equiv.) was added and the mixture stirred for 30 min in reflux. The product was concentrated in vacuum.

Synthesis of (E)-5-(hydroxymethyl)furan-2-carbaldehyde O-benzyl oxime

To a solution of O-benzylhydroxylamine in ethanol (0.1 M), 5-HMF (1 equiv.) was added and the mixture stirred for 30 min in reflux. The product was concentrated in vacuum.

General procedure for imine synthesis

The specific amine was solubilized in methanol (0.1 M), 5-HMF (1 equiv.) was added and the mixture stirred. The reaction was followed by TLC until completion. Solvent was evaporated in vacuum and column chromatography was used for isolation using a mixture of hexane/Ethyl acetate as eluent, if necessary.

General procedure for the synthesis of IEDDA adducts

The specific substrate was solubilized in solvent and the dienophile (2 equiv.) was added to the solution. The reactional mixture stir for 24 h (See Table 1). The product was concentrated in vacuum and isolation was attempted by column chromatography and preparative TLC using hexane and ethyl acetate 8:2.

General procedure for IEDDA reduction

To a solution of the adduct after (Table 1, entry 7 and 8 in $CDCl_3$) in methanol, in 0ºC ice bath, the reducing agent was added in a slow controlled manner. The mixture was allowed to stir for 10 min. water was added to create aqueous phase and extraction followed with ethyl acetate. Water in the organic phase was removed with Na_2SO_4. After filtration, the product was concentrated in vacuum. Isolation was attempted by column chromatography and preparative TLC using hexane and ethyl acetate 8:2.

Procedure for the reduction of furfural

To a solution of furfural (100 mg, 0.9081 mmol) in methanol (0.1 M) in cold bath, $NaBH_4$ (69 mg, 2 eq.) was added. The reaction was allowed to stir for 2 h. quenching with NH_4Cl and extraction with dichloromethane followed. Product was afforded after solvent evaporation in vacuum.

Procedure for acetylation of the reduced furfural

To acetic anhydride (1 mL, 10.59 mmol) was added the total product of the previous described reaction and triethylamine (20 µL, 1 eq.). the reaction mixture was allowed to stir overnight. Extraction with ethyl acetate followed. The obtained solution was concentrated in vacuum.

Procedure for the Vilsmeier-Haack reaction

To a solution of furan-2-carboxylic acid (20 mg, 0.178 mmol) in dimethylformamide (0.14 mL, 10 eq.) with an atmosphere purged with gaseous nitrogen was added $POCl_3$ (18.4 µL, 1.1 eq.). the reaction mixture was sealed with parafilm and stirred overnight. The mixture was then concentrated in vacuum.

Procedure for the Cannizzaro reaction

To an aqueous solution of HMF (100 mg, 1.58 M) was first added $Na_2S_2O_4$.5H2O and then a aqueous solution of NaOH (1.5 M). the reaction stirred for 2h. water was evaporated in vacuum and then ethanol was added. Precipitation was tried with MTBE as the poor solvent.

Procedure for 5-(hydroxymethyl)furan-2-carboxylic acid esterification

To a solution of 5-(hydroxymethyl)furan-2-carboxylic acid (200 mg, 1.4 mmol) in ethanol (7.5 mL, 0.1 M) was added H_2SO_4 as catalyst. The mixture was left in reflux overnight. Quenching with $NaHCO_3$ sat. and extraction with ethyl acetate followed. The organic solvent was evaporated in vacuum.

General procedure for IBX synthesis

To a solution of oxone (3.87 g) in water (16 mL) was added iodo benzoic acid (1.2 g). the mixture was heated up to 73°C and left to stir for 3 h. after this, the mixture was cooled in an ice bath and precipition occurred. The precipitate was the filtrated by gravity and washed with cold water and acetone.

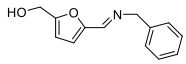
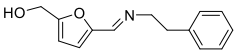
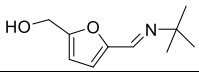
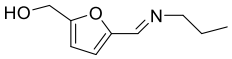
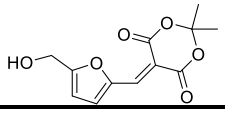
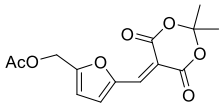
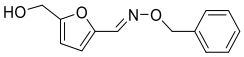
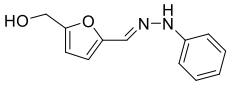
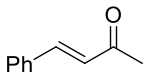
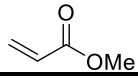
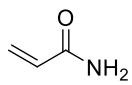
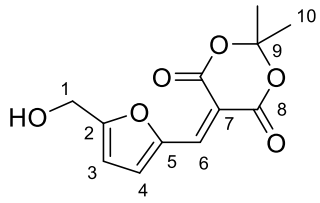
Entry	Specific Substrate	Dienophile	Solvent	Temperature (°C)
1			H_2O	rt
			ACN	75
2			H_2O	rt
			ACN	75
3			H_2O	rt
			ACN	75
4			H_2O	rt
			ACN	75
5			H_2O	rt
			$CDCl_3$	
6			H_2O	rt
			$CDCl_3$	
7			H_2O	rt
			$CDCl_3$	
8			H_2O	rt
			$CDCl_3$	
			$CDCl_3$	
				
				

Table 1 - Resume of reactions in iEDDA adduct synthesis.

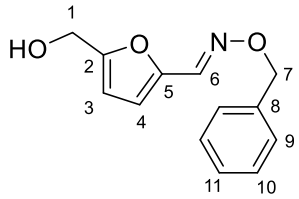
Characterization of the substrates

5-((5-(hydroxymethyl)furan-2-yl)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione



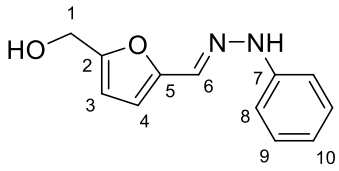
Purification by recrystallization using MTBE and hexane afforded the product as light yellow oil. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 1.75 (s, 6H, H_{10}), 4.75 (s, 2H, H_1), 6.67 (d, *J* = 3.93 Hz, 1H, H_3), 8.28 (s, 1H, H_6), 8.40 (d, *J* = 3.83 Hz, 1H, H_4).

(E)-5-(hydroxymethyl)furan-2-carbaldehyde O-benzyl oxime



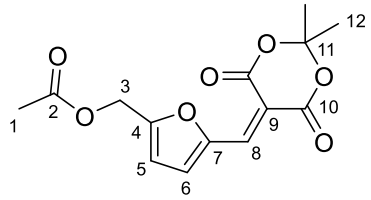
A colorless oil was afforded after solvent evaporation in vacuum. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 4.61 (s, 2H, H_1), 5.51 (s, 2H, H_7), 6.34 (d, *J* = 3.35 Hz, 1H, H_4), 6.54 (d, *J* = 3.34 Hz, 1H, H_3), 7.31-7.43 (m, 5H, $H_{9,10,11}$), 7.98 (s, 1H, H_6).

(E)-(5-((2-phenylhydrazono)methyl)furan-2-yl)methanol



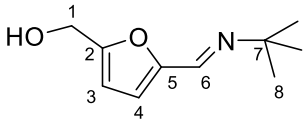
A bright orange oil was afforded after solvent evaporation in vacuum. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 4.63 (s, 2H, H_1), 6.33 (d, *J* = 3.33 Hz, 1H, H_3), 6.47 (d, *J* = 3.35 Hz, 1H, H_4), 6.85 (ddd, *J* = 7.36, 6.06 Hz, 1.16, 1H, H_{10}), 7.07 (dt, *J* = 7.85, 1.16 Hz, 2H, H_8), 7.25-7.28 (m, 2H, H_9), 7.55 (s, 1H, H_6).

(5-((2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)methyl)furan-2-yl)methyl acetate



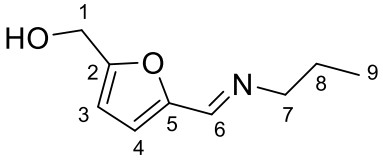
Purification by recrystallization using MTBE and hexane afforded the product as yellow-orange oil. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 1.69 (s, 6H, H_{12}), 2.05 (s, 3H, H_1), 6.67 (d, J = 3.93, 1H, H_3), 8.28 (s, 1H, H_6), 8.40 (d, J = 3.83, 1H, H_5).

(E)-(5-((tert-butylimino)methyl)furan-2-yl)methanol



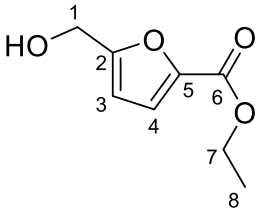
A dark oil was afforded after solvent evaporation in vacuum. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 1.25 (s, 9H, H_8), 4.65 (s, 2H, H_1), 6.35 (d, *J* = 3.34 Hz, 1H, H_3), 6.64 (d, *J* = 3.33 Hz, 1H, H_4), 8.00 (s, 1H, H_6).

(E)-(5-((propylimino)methyl)furan-2-yl)metanol



A dark oil was afforded after solvent evaporation in vacuum. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 0.86 (t, *J* = 7.38 Hz, 3H, H_9), 1.65 (q, *J* = 7.21 Hz, 2H, H_8), 3.45 (dt, *J* = 7.00, 1.33 Hz, 2H, H_7), 4.58 (s, 2H, H_1), 6.29 (d, *J* = 3.35 Hz, 1H, H_3), 6.59 (d, *J* = 3.35 Hz, 1H, H_4), 7.94 (s, 1H, H_6).

Ethyl 5-(hydroxymethyl)furan-2-carboxylate



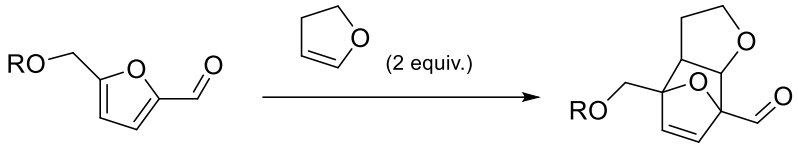
A light yellow precipitate was afforded after solvent evaporation in vacuum. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 1.25 (t, 2H, H_7), 4.23(q, *J* = 7.13 Hz, 3H, H_8), 2.07(s, 2H, H_1), 7.00 (d, *J* = 4.46 Hz, 1H, H_4), 6.30 (d, *J* = 4.46 Hz, 1H, H_3).

4. Results and Discussion

Screening of reaction conditions

In this research project, we aimed to investigate the potential for IEDDA in 5-HMF derivatives and for that we select a readily available molecule with electrodonating characteristics, serving as dienophile: 2,3-dihydrofuran (DHF). This substance is volatile at room temperature and we took advantage of this characteristic to make sure that the possible reaction would occur, using it as an excess reactant.

We first tested the concept to see if the reaction would occur and in what conditions.



Entry	R	Reaction conditions	yield
1	H	DCE, 60ºC	100%
2	H	ACN, rt	N. R.
3	TBDMS	DCE, 60ºC	N. R.
4	TBDMS	ACN, rt	N. R.
5	H	MeOH, rt	N. R.
6	TBDMS	MeOH, 60ºC	N. R.
DCE = dichloroethane, ACN = acetonitrile, TBDMS = tert-butyldimethylsilyl			

Table 2 – Reaction conditions used in screening.

As seen in Table 2, reaction conditions include the screening for solvents (methanol, acetonitrile and dichloethane), at room temperature or with heating (60ºC). The hydroxymethyl group was protected to see if it would affect the reaction. Only the conditions referred in table 2, entry 1 afforded product, indicating that although the reaction was possible, it occurred only in relatively harsh conditions, not suited for biological systems. The hydroxymethyl group was also found to be important, as reaction with the protection group (Table 2, entry 3) produced no results.

The formed adduct was analyzed by ^{1}H NMR. In the spectrum, we can observe two signals corresponding to the newly formed diastereotopic protons, that would only be visible as two duplets after chiral center formation in the adduct, which seems to suggest the formation of the adduct.

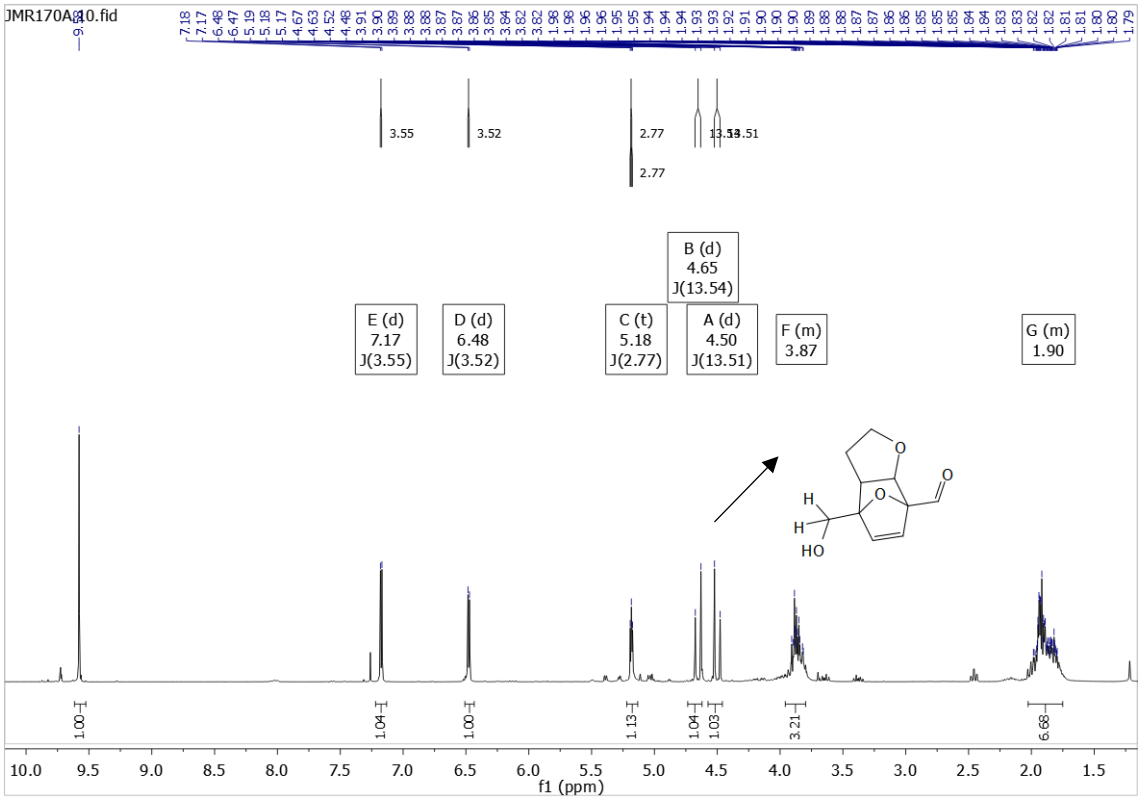
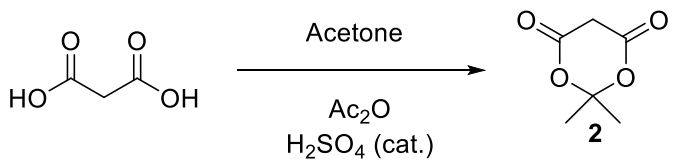


Figure 4 - ^{1}H NMR spectrum of the formed product.

Synthesis of small substrate family

2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid)

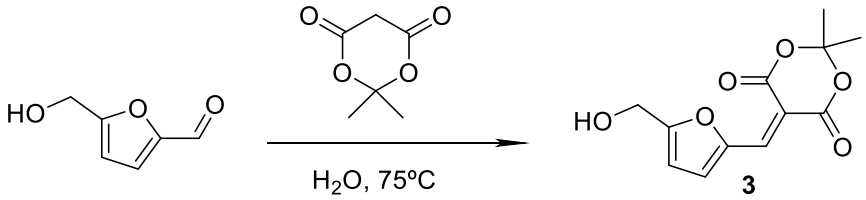


Scheme 4 - General reaction for the synthesis of Meldrum's acid.

In order to synthetize our small substrate family, we needed to obtain first Meldrum's acid. We followed a patent(18) to produce this molecule at an industrial scale which consists in homogenization of reactional mixture comprising acetone, malonic acid and the catalytic sulfuric acid, followed by the addition of acetic anhydride in a slow, controlled manner. This reaction was susceptible to the presence of oxygen, so we used a nitrogen purged atmosphere in the medium, with controlled temperature of about 21ºC. The mixture was allowed to stir for 20h. Quenching followed, adding a saturated aqueous solution of sodium bicarbonate, and then extraction with ethyl acetate. After

concentration in vacuum, no product was found. Looking at the previous steps, we found that after quenching, our reactional mixture had a pH value of 9. Meldrum's acid has a pKa of 4,95 so at pH = 9, the molecule was deprotonated and in the extraction step would not be distributed in the organic phase. After pH adjust to a value of about 4, we obtained product in low yield. Since then, and after a small reaction conditions screening, we used other methods to synthetize Meldrum's acid with high yields, and found that at a laboratory scale, important factors to control are in fact the extend of the quenching process, with final pH value of no more than 4 and also increasing the quantity of acetic anhydride and reduce the reaction time to 4 h increses the yield up to 80%.

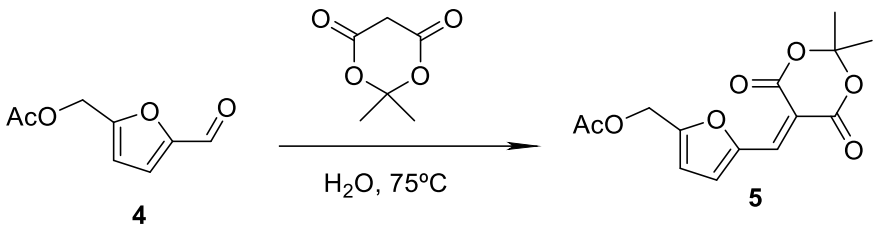
5-((5-(hydroxymethyl)furan-2-yl)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione



Scheme 5 - General reaction for the synthesis of HMF derived product.

With the synthesis of Meldrum's acid achieved, followed the synthesis of the substrates for the inversed demand Diels-Alder. We started with the reaction of 5-HMF with Meldrum's acid, using water as solvent at 75ºC. After extraction with dichloromethane, the separation and purification of the product was attempted by chromatography in a silica column, using a mixture of hexane and ethyl acetate as eluent in proportions of 7:3 and 6:4. The results were disappointing, and no separation was achieved. Recrystallization method was attempted using as rich solvent MTBE and hexane as poor solvent. Although in low yields, purification was achieved, but the process had to be repeated several times until HMF was eliminated as a contaminant. In following attempts, this reaction was conducted at low temperatures of about 65°C. This new reaction conditions afforded better results for the product would precipitate and allowed filtration to followed.

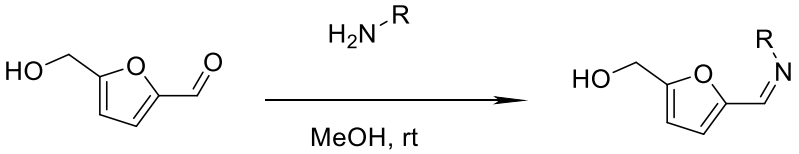
2,2-dimethyl-5-((5-(2-oxopropyl)furan-2-yl)methylene)-1,3-dioxane-4,6-dione



Scheme 6 - General reaction for the synthesis of Meldrum's acid product with acetalized HMF.

It would be pertinent to alter not only the aldehyde end of 5-HMF, but as well as the hydroxymethyl end, in order to study the effect of this type of substitution in the IEDDA. To achieve that purpose, we reacted acetilated HMF, previously synthetized in our laboratory, with Meldrum's acid. The reaction conditions were similar, using water as solvent at 75ºC. To purify this product, we first attempted the recrystallization method with MTBE and hexane, since this process worked relatively well in the previous case. No product precipitation was observed, even after overnight rest in low temperature. The second approach was separation and purification trough preparative thin layer chromatography. As eluent, we used a mixture of hexane and MTBE in the proportion of 8:2. We allowed the eluent to run a second time to achieved better separation and after the process was completed, product was obtained in low but usable yield.

Imines



Scheme 7 - General reaction scheme for the synthesis of amine derived products.

Most our small substrate family comprises structures resulting from 5-HMF reaction with several amines previously synthetized and isolated in our laboratory (Figure 5).

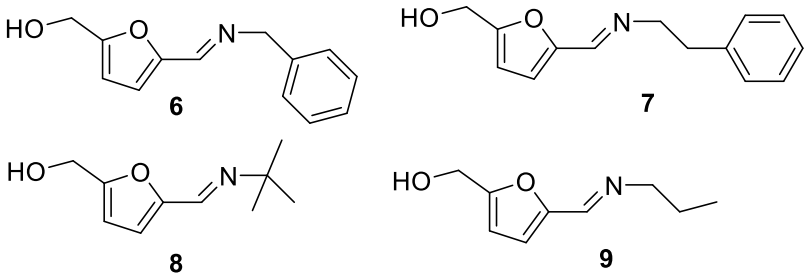


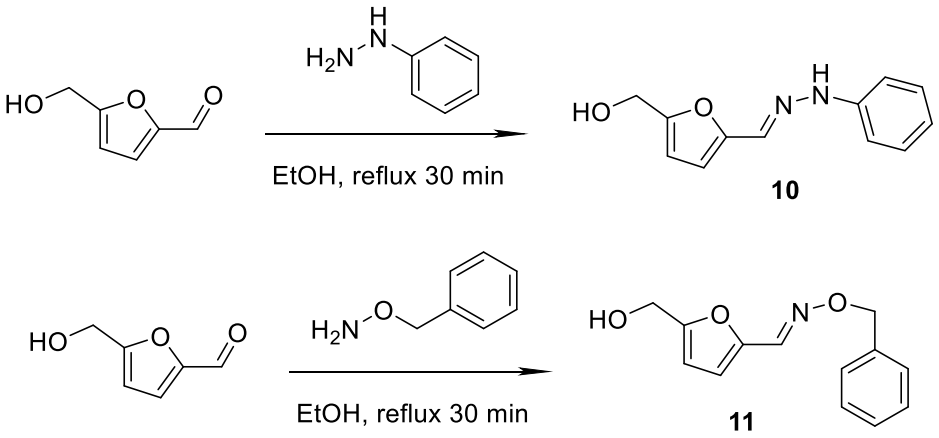
Figure 5 - Previously synthetized 5-HMF derived imines.

Hydrazone and Oxime

The imine formation was quantitative, however the hydrazone and the oxime presented impurities. The most promising substrates for the IEDDA were these structures, due to their strong electron withdrawing groups. Although the molecules were synthetized, they needed purification. The first approach used was separation and purification trough chromatography in a silica column, but once more degradation was observed. For the (E)-5-(hydroxymethyl)furan-2-carbaldehyde O-benzyl oxime, a preparative TLC was attempted using as eluent a mixture of hexane and MTBE in a proportion of 1:1. The method produced no product. Since the starting samples were of low quantity and the processes used in the attempt of isolation result in high losses and degradation products, the purification approach was abandoned, and a new, clean synthetic process was suggested. In order to achieve this, we first tried the synthesis of (E)-(5-((2-benzylhydrazono)methyl)furan-2-yl)methanol trough reaction of HMF with benzylhydrazine, using methanol as solvent at room temperature, since those were the conditions used in the preparation of the imine substrates. The results were disappointing, with the process leading to the presence of several contaminants that would have to be separated trough chromatographic methods that previously were not successful. Therefore, we decided to look in the literature(19–21) for other ways to synthetized this compounds. We select three approaches summarized in Table 3. The reaction with benzylhydrazine was previously unsuccessful, so we tried to use phenylhydrazine, with much better results. After screening different conditions, the best was clearly using ethanol as solvent, heating in reflux for 30 min. The reaction was quantitative with no need for extraction, making it the cleanest method.

Reaction conditions	yields
Acetonitrile, buffer pH=4, rt (0,1M)	≈ 100%
Ethanol, reflux 30 min (0,1M)	≈ 100%
Methanol, sodium acetate (1 equiv.), rt (0,1 M)	No reaction

Table 3 - Reaction condition used for optimization of 5-HMF derivatives.



After all the obstacles were surpassed, we end up with the desired substrate family (Figure 6). At this staged we realized that although HMF is a valuable and versatile molecule, it presents some issues, namely the difficulty of separation from the desired products. Amongst other factors, its melting point at room temperature presents problems in purification methods such as recrystallization. One thing we notice is that when 5-HMF precipitates it forms an emulsion-like mixture that covers the other precipitated products with a thin film layer, making it extremely difficult for separation to occur. This phenomenon is known by "oiling out" or liquid – liquid phase separation and is governed by variables like concentration and temperature in the recrystallization process(22). Adjusts were made in order to account for this fact and generally afforded better results, but separation continued to be a difficult process. Regarding the substrates *per se*, they too present some issues. Common methods used in isolation don't seem to be useful, probably because they use silica as a stationary phase for separation and these molecules seem to be unstable in silica, resulting in various degradation products.

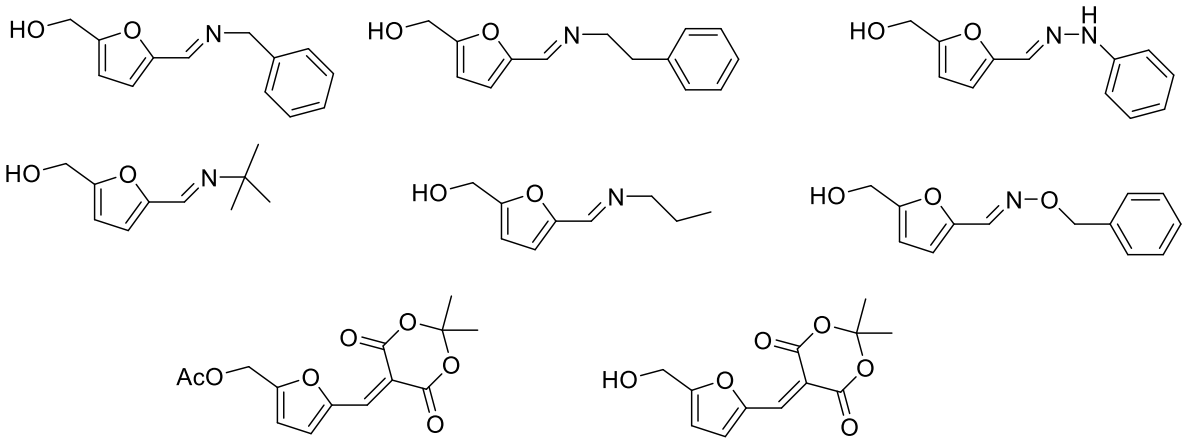
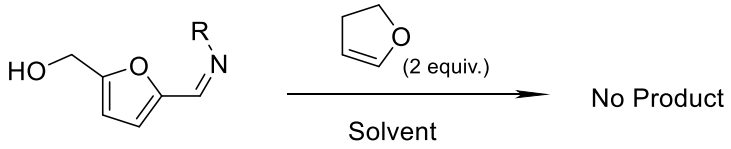


Figure 6 - Small substrate family.

IEDDA reaction

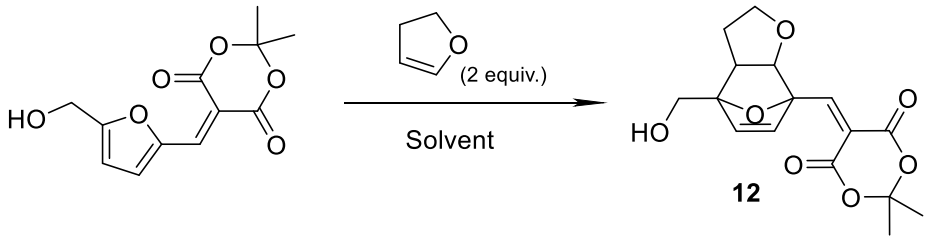
Imines derived from HMF



Scheme 8 - General reaction for the synthesis of anime derived adducts.

For these reactions to become relevant in biological context, the concept had to be tested using water as solvent. We started the screening process using the imine derivatives in water (0,1M) and adding 2,3-DHF, sealing the reaction medium with parafilm, in order to prevent 2,3-DHF evaporation. The mixture was allowed to stir for 24 h at room temperature. after TLC analysis, we concluded that no reaction took place and decided to provide the reaction medium with temperature, this time using DCE as solvent. Once more no reaction took place.

The Meldrum's acid conjugate

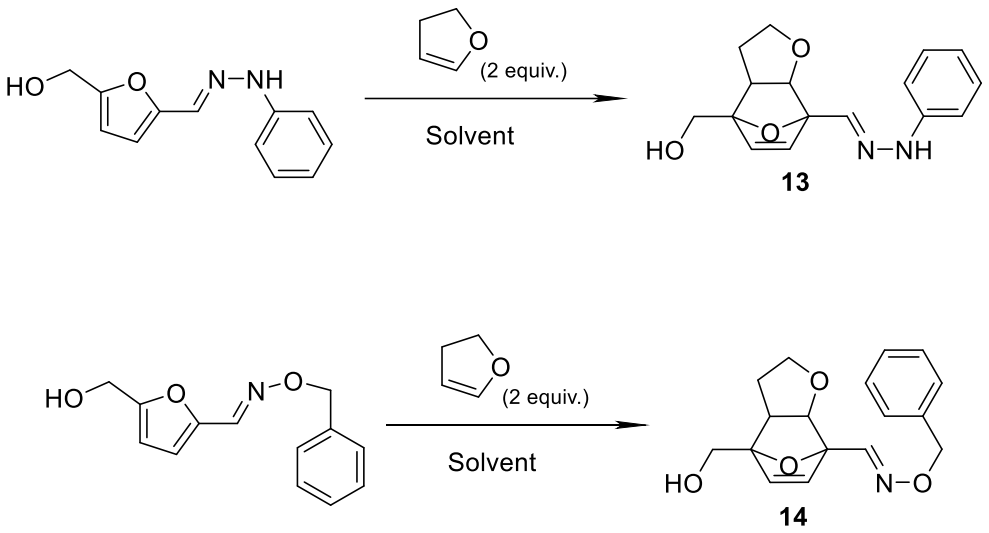


Scheme 9 - General reaction for the synthesis of Meldrum's acid adduct.

From previously data, we knew that this substrate had high potential to react. An aqueous solution (0,01M) from the substrate was prepared and after 10 min of the addition of 2,3-DHF, a small uncolored precipitate was observed. After this promising result, the reaction was followed by ^{1}H NMR.

Synthesis and isolation was attempted, using column chromatography and preparative TLC but no separation was achieved, although theoretically in chloroform the reaction affords 100% in yield and separation is not needed.

The hydrazine and oxime adducts

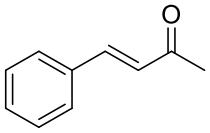
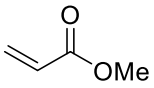
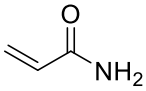


Scheme 10 - General reaction for the synthesis of the hydroxy inime and hydrazine adducts.

Following the relatively successful attempted with 5-((5-(hydroxymethyl)furan-2-yl)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione substrate, we use the HMF derived oxime and hydrazone in a similar method, using water:acetonitrile as solvent (0,01M) in order to follow the possible transformation in HPLC. We observed a formation signal for the adduct, but when we took the reaction to ^{1}H NMR no product was observed. Instead, we saw spectrum signals that seemed to indicate the presence of the IEDDA adduct from the reaction of HMF and 2,3-DHF as well as the original hydrazine. This led us to conclude that these types of structures are unstable and readily hydrolyzed. We observed some problems related with adduct solubility, often needing more solvent to achieve complete solution, even in polar solvents like chloroform.

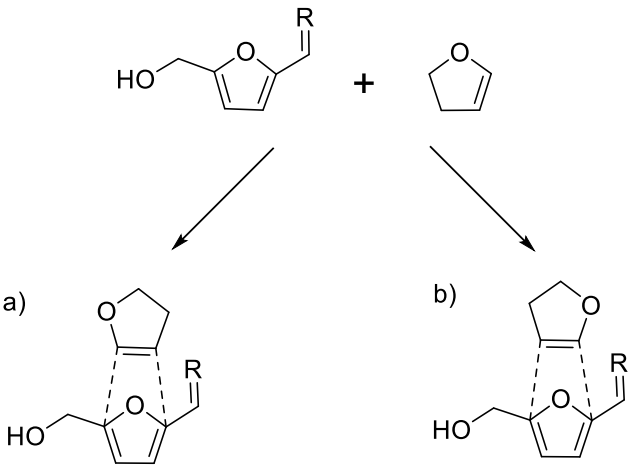
Using other dienes

After the first unsuccessful attempts at adduct formation, we wonder if the problem was in the diene used. Perhaps these types of substrates would react faster in a normal DA route. In order to test this hypothesis, we select three new molecules to serve as dienophiles (Table 4), this time with electronwithdrawing groups. No reaction took place with these new reactants, so we conclude that a normal DA approach was not viable.

Diene	Yield
	No reaction
	No reaction
	No reaction
Reaction conditions: chloroform (0,1M), rt; dienophile (2equiv.)	

Considerations about adduct stereochemistry

As previously cited, Diels-Alder reactions tend to produce the *endo* product. Since none of the final products were evaluated properly to show this kind of specific stereochemistry, we can only assume that the tendency is followed (Figure 11). Regarding the orientation of 2,3-DHF, there are two reaction possibilities: the adduct can be formed with the ether group of 2,3-DHF in line with the hydroxymetyl group of the HMF derivatives (Scheme 11, a)), or in line with the aldehyde substituted group of HMF derivatives (Scheme 11, b)). We can theorize that the p orbital in the O atom in 2,3-DHF interacts with the non-ligand π orbit in the HMF derivatives to produce the latter option.



Scheme 11 - Possible reaction possibilities.

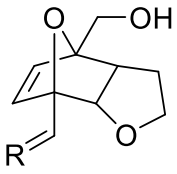


Figure 11. *Endo*-adduct.

IEDDA adducts reduction

One possible solution for the degradation problem observed with the IEDDA adduct was attempting its reduction using $NaBH_4$ and $LiBH_4$, theoretically leading to more stable structures. After adduct formation, solvent was evaporated in vacuum and the remaining precipitate solubilized in methanol. After the addition of $LiBH_4$, product was extracted using ethyl acetate as organic phase. The isolation was attempted by preparative TLC. Although separation was achieved, no product was isolated, leading us to conclude that the reduction process was unsuccessful.

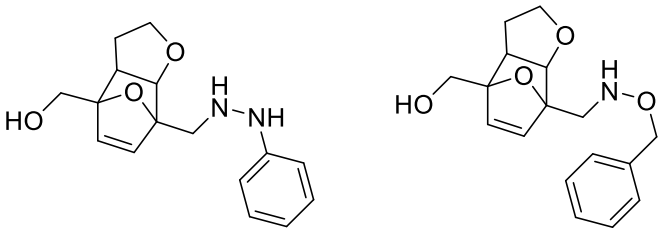
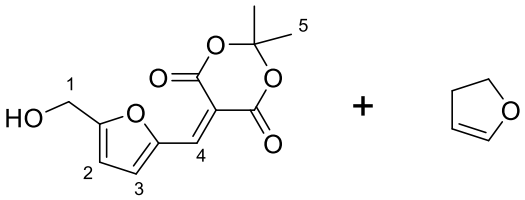
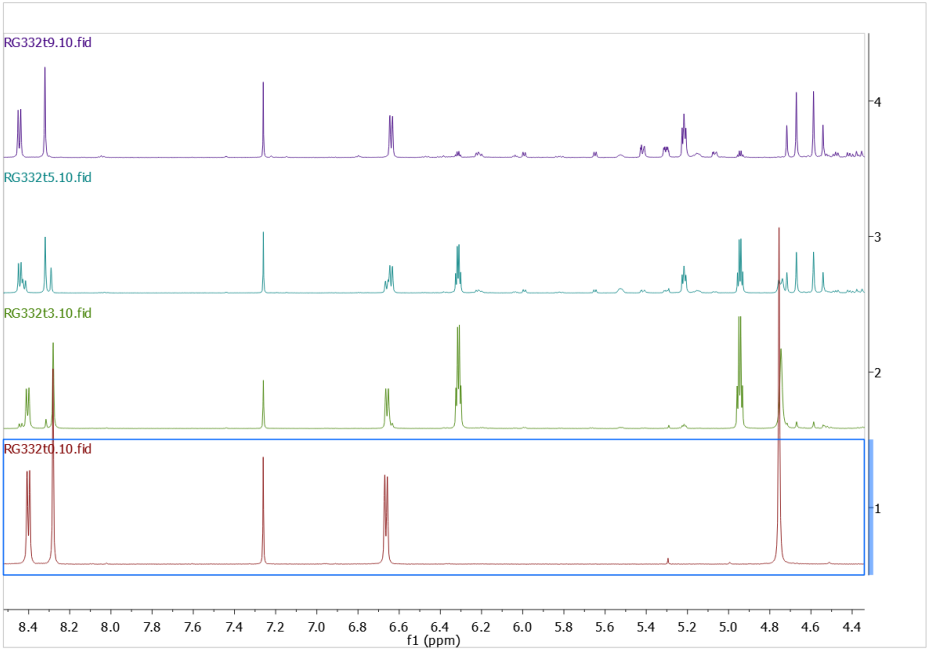


Figure 10. Expect structures after reduction process.

Following adduct formation trough ^{1}H NMR

As stated previously, none of the final adduct products were isolated although several attempts were made. Nevertheless, the reaction of some of the most promising substrates were followed by ^{1}H NMR. The general procedure for this process was to have a substrate solution in $CDCl_3$ that was analyzed, marking the t_0 of the experiment, and after that the DHF was added to the solution and the sample analyzed in several time periods. The collected data follows.





The experiment with Meldrum's acid conjugated substrate showed some interesting transformations occurring in the molecule. The pick at 4.76 ppm (Figure 12 c)), initially a singlet, unfolds into a pair of doublets, information that is in line with the previous observations in the original screening, indicating once again the formation of diasteriotopic protons in site 1 and the formation of a chiral center in the molecule. Picks corresponding to protons 3 and 4 at 8.40 and 8.28 ppm (Figure 12 a)) respectively shifted slightly downfield as picks corresponding to protons 1 and 2 at 4.76 and 6.66 ppm (Figure b)) shifted slightly upfield which could indicate a symmetry axis, the furan ring, in which the transformation occurred, changing the protons environment. All this data seems to support the DA adduct formation.

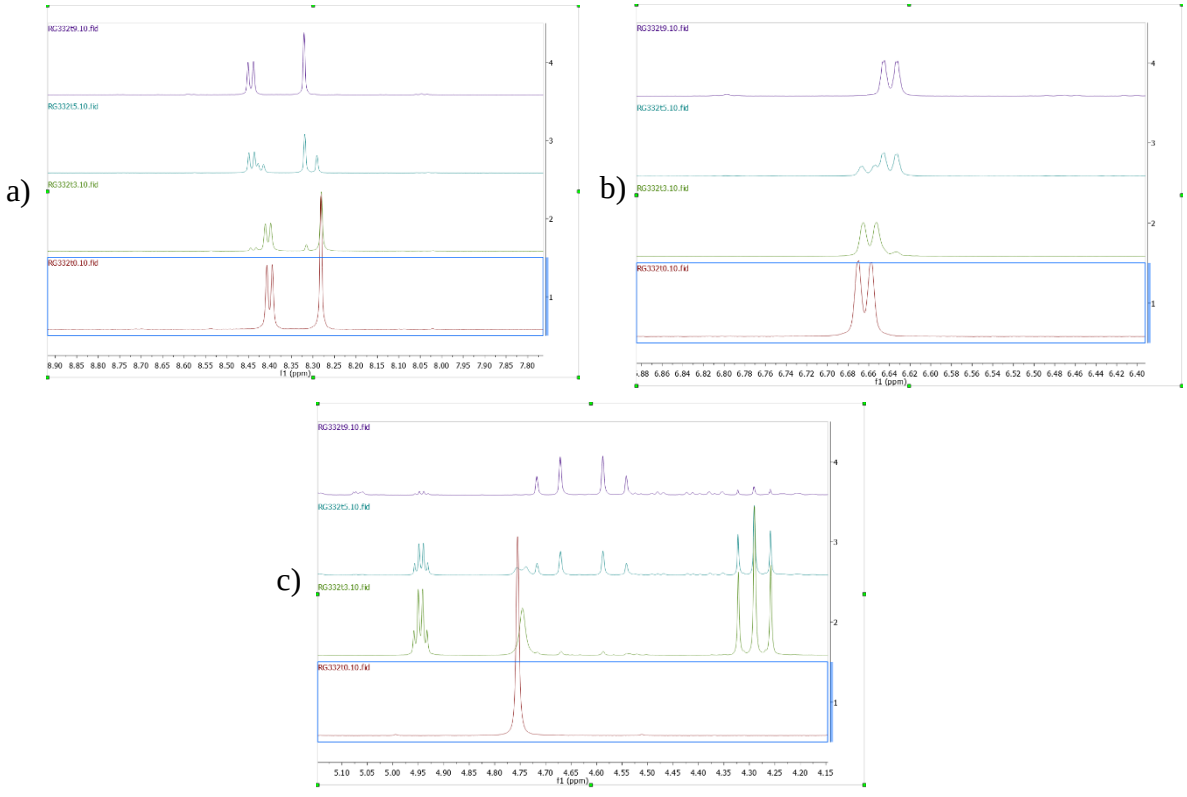


Figure 12 – Close ups of 1H NMR spectra of the Meldrum's acid conjugate. a) protons 3 and 4, b) proton 2, c) proton 1.

Similar transformations occurred when the oxime was used (Figure 13). Diasteriotopic protons were also formed and pick shifting occurred, once again ensuing modifications to the molecule that seems to support the theorized adduct formation.

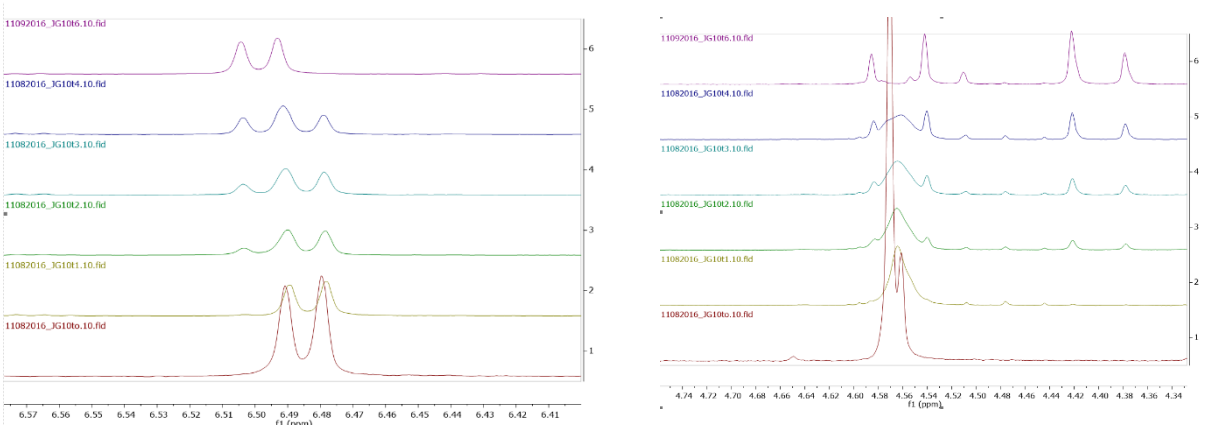
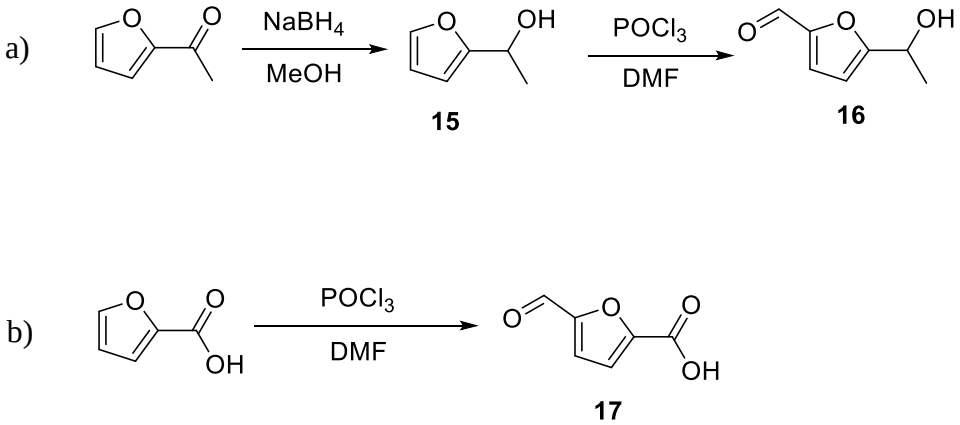


Figure 13 –1H NMR spectra of the oxime.

Unfortunately, all samples showed degradation at the end point and isolation of the final product was never achieved.

New substrates for iDDA: the aldehyde ester substrate

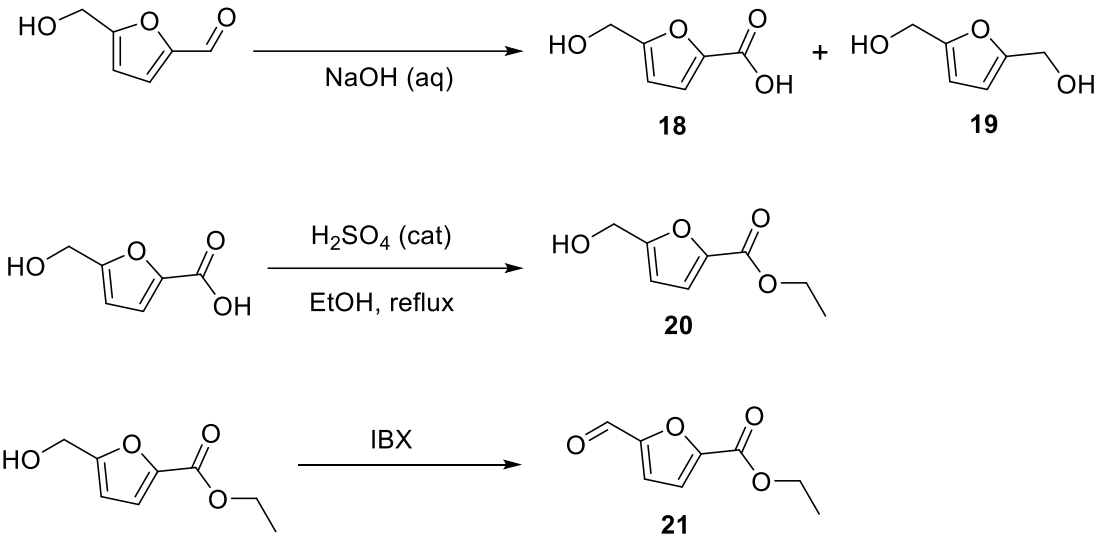
Following the relative success of the previous attempts, we considered new substrates for the reaction, trying to solve the isolation problems and collect more definite that would help elucidate the observed product. A good candidate would be a substrate with an end in the formyl form and the other with an ester form, two strong electronwithdrowing groups, more inert to degradation and with better solubility profiles. The first tried approach was to start from a few furfural based molecules and attempted a Vilsmeier-Haack reaction, formylating the furan ring (Scheme 11). This transformation was described for similar species in the literature with a 20% yield(23).



Scheme 11 – Vilsmeier-Haack reaction attempts.

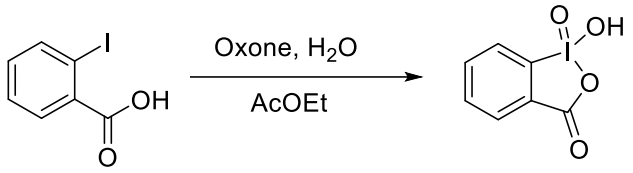
The attempt a) (Scheme 11) started with a simple reduction with sodium borohydride and after extraction and solvent evaporation the process afforded a light yellow liquid. In order to prepare the product to 1H NMR analysis, the sample was left in a vacuum bomb overnight. After this step the sample showed degradation, turning into a dark green oil. Following attempts were not successful and as the molecule 15 was never isolated, the Vilsmeier-Haach reaction was never attempt. We tried again starting with the furan-2-carboxilic acid (Scheme 11 b)) but after checking the end point trough TLC it was obvious that no reaction took place.

Another approach was devised starting from the HMF itself. Through a Cannizzaro reaction, a kind of organic reduction-oxidation chemical transformation, HMF would lead to the respective carboxylic acid 18, as well as the dihydroxymethylalcohol 19, and from 18, an esterification process would occur, affording 20. If this desired molecule was afforded, an oxidation would follow using IBX, resulting in the aldehyde ester substrate 21 (Scheme 12).



Scheme 12 – Reaction scheme for obtaining the aldehyde ester substrate.

From the initial step, we encountered several problems. The Cannizzaro reaction was not as successful as thoughted, the final product was obtained in low yields and with considerable amounts of degradation products. Using the molecule 18 previously synthetized in the laboratory by other methods, we achieved a relatively successful esterification, with low yields but good purification. The final oxidation step was tried but the reaction was not quantitative as expected and a chromatographic column was needed. With all said and done, the final product 21 was not afforded in useable yields and this substrate was not subjected to the iEDDA reaction.



Scheme 13 – Obtaining IBX.

Furfural and its Stenhouse salts

Another interesting chemical feedstock that can be obtain from biomass is furfural. Much like HMF, furfural is already use as an intermediate in the synthesis of important molecules such as furfuryl alcohol and tetrahydrofurfuryl alcohol used in polymer and herbicide chemistry, as well other furan chemical and furan itself and its even used as a polar chemical solvent(24)(25). One of the synthetical transformations for furfural is the formation of Stenhouse salts. These are stable, ring opened, intensely colored structures formed by first, an initial ring activation of furfural by, for example, an imine formation in the formyl group and then a ring attack that results in the open desired molecule. Under some specific circumstances, mainly under visible light and in non chlorated solvents, some of these molecules open form can be turned into a cyclopentenone form, a property that is being explored it permits a light controlled switch between the two presentations(26). However, looking at the open form, we can detect a conjugated π system capable of a *cis* conformation that might allow for a DA reaction to occur (Figure 14). Bearing this in mind, we experiment with two Stenhouse salt derived from furfural conjugated with Meldrum's acid.

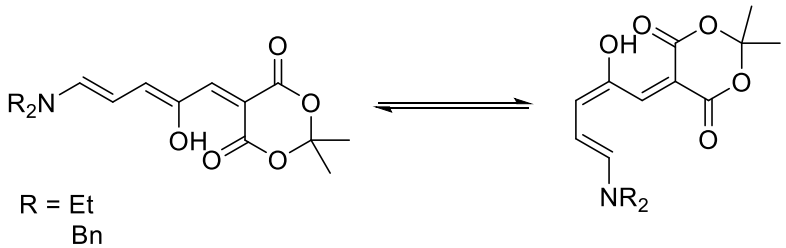
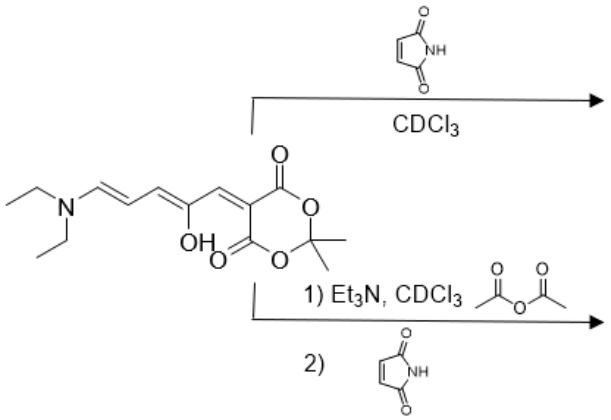


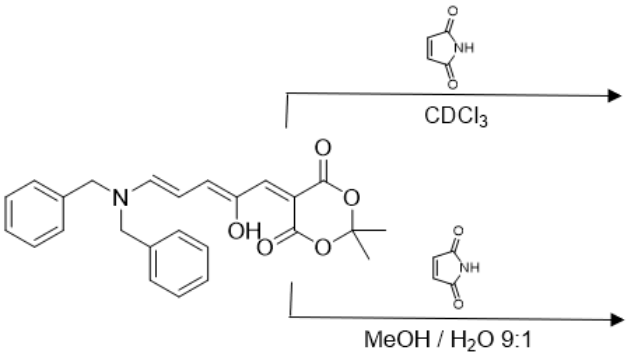
Figure 14 – *trans* and *cis* forms.

As stated previously, this Stenhouse structures tend to cyclize when expose to visible light. In order to stop this phenomenon, we tried to protect the alcohol "hijacking" the non-ligand electrons in the hydroxyl group responsible for the cyclization with silane protective groups such as TMSCl and TBSCl, but none of the attempts were successful. We followed a simpler approach in which the protection with acetic anhydride would be followed with the immediate reaction with maleimide, a known diene capable of DA reaction, this time the normal type. Reaction without the protection step was also attempted, but both tries afforded no palpable results (Scheme 14).



Scheme 14

Another approach was made by using a Stenhouse salt incapable of cyclization, were the protection step was not needed (Scheme 15)). The reaction was also conducted in a MeOH/H_2O mixture which would promote the DA reaction bout once again the process showed no results.



Scheme 15

5. Conclusion and future perspectives

Although original screening only produced adduct formation in harsh conditions, not suited for biological systems, further investigation revealed that this type of reactivity occurs with decent rates in biological conditions as long as the 5-HMF derivatives substrates have strong electrowithdrawing groups. Solubility of this substrates is an issue that needs address and new modifications are being made in order to improve structure solubility. In the future we hope to test these types of reaction with more efficient dienophiles. In resume they are a possible reliable method for biological and pharmaceutical applications.

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